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Experimental Simulation of Microcystis Bloom Formation from Natural Phytoplankton Communities

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Abstract

An experiment with easy, repeatable management was carried out to demonstrate *Microcystis* bloom formation from natural phytoplankton communities. This was achieved in warm season by putting eutrophic, high chl *a* content water with a natural phytoplankton assemblage (no cyanobacteria bloom) containing *Microcystis* into colorless transparent glass jars outdoors for a few days and then with nutrients enrichment. In the experiment, water of 3.5-4.5 mg/L TN, 0.3-0.4 mg/L TP and 120-160 μg/L planktonic chl *a* from a pond was put into two jars on 12 June 2007. On 25 June, net addition of 3.1 mg/L NO_3-N and 0.45 mg/L PO_4-P was put into one jar, and by 4 July, a *Microcystis* bloom had occurred on the surface water of the enriched jar, but not in the other. On 5 July, the nutrients addition was repeated, and subsequently the *Microcystis* bloom became more significant. On 8 July, the same concentration of nutrients was added to the remaining jar, and by 10 July, a *Microcystis* bloom had also occurred in the second jar. The nutrients enrichment provides a chance for the *Microcystis* out-competing the other algae from natural eutrophic water rich of phytoplankton. The experiment demonstrates an easy and repeatable method of achieving a *Microcystis* bloom from natural phytoplankton communities, which may be useful for the further study of bloom mechanisms.

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Keywords: Microcystis bloom; Phytoplankton; Nutrients; Experimental simulation

Introduction

Eutrophication and algal blooms–especially *Microcystis*–blooms have been occurring in lakes worldwide[1,2] and have been a strong research focus for lake ecosystems. However, the knowledge for the mechanisms of algae blooms is inadequate, and the blooming process is not clearly understood.

There are some reasons for it. Firstly, it has proved difficult to get colony formation rather than single cells when culturing phytoplankton under lab conditions[3,4] and it is very difficult to achieve algae

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blooms under lab condition in order to study the bloom processes. Even though colony formation of some phytoplankton is triggered by 'infochemicals' released during the grazing process[4-6], it isn't enough for bloom formation. The characteristics between laboratory *Microcystis* populations and natural *Microcystis* populations are different from each other and they can response differently to solar irradiance[7]. Moreover, *Microcystis* exists in colony rather than in single cell in natural water with no bloom. Large colonies formation does not equate to bloom formation, and their mechanisms may be not the same

Secondly, although *Microcystis* bloom formation has always been linked to eutrophication[8], it doesn't only occur in eutrophic lakes, but also meso-eutrophic lakes, e.g. Lake Qiandaohu of China in 1998 and 1999[9], and not all eutrophic lakes experience algae blooms. Many studies[10-13] have addressed the relationship between nutrient supply, nutrient ratios and phytoplankton composition, but haven't found an assured mechanism for algal bloom due to eutrophication. In searching for clues to explain the occurrence and abundance of gas-vacuolate cyanobacteria, efforts have been made to identify adaptive advantages that they might have over competing eukaryotic micro-algae, e.g. gas vacuoles, light capture ability, nitrogen fixation, sunscreen pigments[2], but it is still an issue of contention.

During the former studies, the occurrence of algal bloom was not considered reliant on any one particular environmental stimulus, but depended on a complex interaction of factors, including the unique characteristics of each bloom-forming cyanobacteria species, and exogenous (environmental) factors[2]. The relationship of environmental characteristics supporting the development of cyanobacterial bloom are so complicated that how these interactions finally drive the bloom to occur was unknown. So it is very important to find a simple and effective method to recur algal bloom in more controllable condition than the field. It is very possible that the environmental differences between lab incubation and field situation make algal bloom occur.

Usually, cyanobacteria blooms are inclined to happen during warm seasons, and temperature plays a key role, with reservoirs in subtropical and tropical regions having more sustained annual blooms of cyanobacterial species than temperate regions[14]. Jacoby et al.[15] thought the bloom occurrence has a relationship to increasing water temperature and high nutrient content.

By analyzing so many factors which may affect bloom formation and the responses of phytoplankton, we tried and found a simple experimental simulation of making algal bloom occur in our experiences.

1. Materials and methods

This experiment was conducted outdoors during early summer from June to middle July, 2007. The experimental water was obtained from a 300 m^2 pond with no algal bloom. The water depth of the pond was about 60 cm with Secchi depth of about 25 cm during the experiment time. .

The water was put into two rectangular transparent colorless glass jars (Jar A and Jar B) of 30cm*25cm*40cm each on 12 June 2007. On 25 June, 10 mL of a nitrogen and phosphorus solution, made by dissolving 8.3108g KNO_3 and 0.7220g KH_2PO_4 into 200 mL distilled water with the N/P (weight ratio) ratio of 7:1, was added to Jar A. On that day, the water volume was 22 L, therefore, the net increase was 3.1 mg/L NO_3-N and 0.45 mg/L PO_4-P. On 5 July, a further 10 mL of the same nutrient solution was added to Jar A. On 8 July, 10 mL of the nutrient solution was added to Jar B. During the experimental period, no distilled water was added to the jars and the water volume of each jar was about 18.5 to 22 L.

Water samples were analyzed for total nitrogen (TN), total phosphorus (TP), total dissolved nitrogen (DTN), total dissolved phosphorus (DTP) and chlorophyll *a* (chl *a*). Water for DTN and DTP analyses was filtered through 0.45 μm polycarbonate filter. Water temperature was measured at 08:00 a.m. and 14:00 p.m. For TN, TP, DTN and DTP analysis, water samples were digested by alkaline potassium persulphate in a high pressure sterilization pot at 120 ^oC. Nitrogen was measured directly with

spectrophotometer and phosphate concentration and SRP were determined by molybdenum-antimony-ascorbic acid colorimetry.

To determine chl *a* concentration, water samples were filtered through GF/C (1.2 μm pore size) filters (Whatman, Maidstone, U.K.). Chl *a* concentration was determined by colorimetry after the residue was extracted by 90% ethanol[16,17]. Phytoplankton samples had 100% Lugols solution added to give a final concentration of 1%, and samples were stored in the dark until identification. In the laboratory, samples were identified to genera level under a Zeiss microscope (Germany)

2. Results

The water temperature's variation of the two jars (Fig. 1) showed the temperature in the afternoon was mostly higher than 26.0 ^{o}C with a mean of 30.7 ^{o}C. The mean water temperature in the morning was 25.5 ^{o}C, which was usually lower than the afternoon

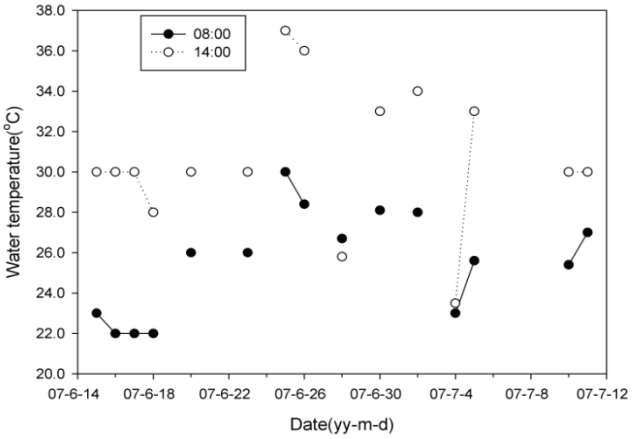

Fig.1 The variation of water temperature of the two jars at 08:00 a.m. and 14:00 p.m. from 15 June to12 July, 2007

The background nutrient and chl *a* is given in Table 1, showing that the concentrations of TN, TP, DTN and DTP in Jar A and B on 10 July had greatly increased since 20 June. The genera composition of phytoplankton in the pond during late May to early June (and the initial water for the jars) was *Synedra*, *Navicula* and *Attheya* of Bacillariophyta; *Chlamydomonas*, *Cosmarium*, *Scenedesmus*, *Crucigenia*, *Ankistrodesmus*, *Golenkinia* and *Coelastraceae* of Chlorophyta; *Euglena*, *Trachelomonas* and *Phacus* of Euglenophyta; *Aphanocapsa*, *Microcystis*, *Merismopedia* and *Synechocystis* of Cyanophyta; *Synura* and *Dinobryon* of Chrysophyta. Cyanophyta was visually dominant in individuals and cells; however, *Microcystis* was not dominant.

Table 1 The background water quality of the pond and the two jars during the experimental time

Parameter	Pond on 27 May	Pond on 3 June	Pond on 8 June	Pond on 20 June	Jar A on 20 June	Jar B on 20 June	Jar A on 10 July	Jar B on 10 July
TN(mg/L)	3.326	3.400	3.867	4.526	3.853	4.676	7.111	5.847
TP(mg/L)	0.245	0.213	0.278	0.404	0.317	0.393	0.932	0.690
DTN(mg/L)	1.283	0.927	0.924	0.608	-	-	4.024	3.129

DTP(mg/L)	0.074	0.042	0.020	0.209	-	-	0.295	0.164
TN/TP	13.5	15.9	13.9	11.2	12.1	11.9	7.6	8.5
DTN/DTP	17.4	22.3	45.8	2.9	-	-	13.6	19.1
Planktonic chl *a*(μg/L)	-	118.11	158.64	-	-	-	-	-

Notes:In the table, "-" means the value wasn't measured.

Although the proportion of each genera was not quantified for the phytoplankton in the pond and the two jars, a visible *Microcystis* bloom occurred at the surface of Jar A on 4 July, and it became more significant on 11 July. No *Microcystis* bloom occurred to the surface water of Jar B until 10 July. Meanwhile, no *Microcystis* bloom occurred in the pond over the entire duration of the experiment.

3. Discussion

A *Microcystis* bloom occurred in Jar A following both the first and second nutrient additions, but not in Jar B before 8 July. It is believed phytoplankton need more nutrients when the water temperature is above optimum[18-20]. Therefore, although the TN and TP content didn't changed greatly on 20 June with high temperature and strong illumination, the phytoplankton needed more nutrients to maintain growth. Following nutrient addition, the water turned much greener, even before the *Microcystis* bloom was obvious. After nutrient addition to Jar B on 8 July, a *Microcystis* bloom occurred several days later. Nutrients were added to Jar A earlier than to Jar B, and the *Microcystis* bloom also occurred earlier. This shows that nutrient addition can drive *Microcystis* blooms under this experimental design.

Therefore, nutrient addition plays an important role in algae bloom occurrence, and it is possible to create *Microcystis* blooms from natural water with natural phytoplankton compositions under our more controllable conditions than field conditions. Furthermore, it can be concluded that if no nutrient addition was made to either jar, *Microcystis* bloom would not have occurred in either. In this experiment, the added nutrient was at least NO_3-N 3.1 mg/L and PO_4-P 0.45 mg/L for each addition–levels which must be over the critical concentration for bloom formation using these experimental conditions

A lot of studies[10-13,21] have been conducted regarding the effects of nutrient addition on total phytoplankton and composition, using different experimental designs to that presented here. Although the mechanisms of *Microcystis* bloom formation are very complicated, our simple experimental design does result in a *Microcystis* bloom. Ours was creative in putting water rich of natural phytoplankton community into transparent colorless glass jars outdoors in warm seasons with nutrients addition a few days later. Rhew et al. [22] studied the interaction of fish, nutrients, mixing and sediments on autotrophic picoplankton and algal composition, and different algal blooms were observed at high nutrient treatment with different interactions. However, no cyanobacteria bloom formed, and the authors suggested the lack of a cyanobacteria bloom may have resulted from low water temperature. This kind of interaction makes it hard to explain why and how the bloom occurred, but an important point is that algal blooms occurred at high nutrient conditions.

Based on the occurrence of a *Microcystis* bloom after nitrogen and phosphorus addition, a hypothesis formed: once the high phytoplankton-containing water was transferred into transparent glass jars, the high water temperature, strong illumination, and inadequate active nutrient content couldn't maintain the well growth of all the phytoplankton, and the addition of sufficient active nutrient later made *Microcystis* out-compete the other algae. And the *Microcystis* bloom formed. The high content nutrient addition is the most important driver of bloom formation.

Cyanobacteria blooms occur often in summer with high temperature, which shows high temperature and strong sunshine contribute to algal bloom formation. Under our experimental design, the

discontinuous but large quantity of nutrient addition made *Microcystis* blooms occur quickly. In shallow eutrophic lakes, the nutrient-rich sediment can release nutrients to the overlying water as intrinsic nutrient source. Intrinsic and extrinsic nutrient source makes the nutrients support as discontinuous and in large content as our experiment here, and the stimulating factors and suppressive factors interplay without temporal separation. So their roles are not as obvious as our experiment here.

It is certain that in a blooming water body, the nutrient condition for algal bloom occurrence is enough and it interacts with other environmental factors simultaneously, making the algal bloom perplexing and mysterious. One more problem is that not all eutrophic water has algal bloom, while the *Microcystis* bloom of Donghu Lake, Wuhan, China, disappeared[23] after its appearance for decades. This suggests that nutrients supply is just one condition necessary for algal bloom. And the suppressed environmental factors such as high water temperature, strong irradiance and deficiency of nutrients for all the phytoplankton, are the preconditions. Because of its unique characteristics[2], *Microcystis* can endure difficult conditions. Once enough nutrients are provided at a later time, *Microcystis* can form blooms in the suppressed environment. And our further experiment found that the selective grazing from zooplankton help *Microcystis* to out-compete the other algae, especially green algae[24].

Summary

A simple method of simulation of Microcystis bloom formation was introduced here, which refers to enough nitrogen and phosphorus addition to the eutrophic water with natural phytoplankton community of above 100 μg/L chlorophyll-a content in warm seasons outdoors

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